Proximate Developmental Mediators of Sexual Dimorphism in Size: Case Studies from Squamate Reptiles

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Synopsis

Sexual dimorphism in size (sexual size dimorphism; SSD) is nearly ubiquitous, but the relative importance of genetic versus environmental control of SSD is not known for most species. We investigated proximate determinants of SSD in several species of squamate reptiles, including three species of *Sceloporus* lizards and the diamond-backed rattlesnake (*Crotalus atrox*). In natural populations of these species, SSD is caused by sexual differences in age-specific growth. Males and females, however, may often share similar potentials for growth: growth is strongly responsive to the availability of food, and sexual differences in growth can be greatly suppressed or completely absent under common environmental conditions in the laboratory. Sexually divergent growth is expressed in natural environments because of inherent ecological differences between males and females and because of potential epigenetic effects of sex-specific growth regulators. In field-active *Sceloporus*, sexual differences in growth rate are associated with sexual divergence in plasma testosterone. Experiments confirm that testosterone inhibits growth in species in which females are larger (for example*, S. undulatus* and *S. virgatus*) and stimulates growth in those in which males are larger (for example, *S. jarrovii*). Interestingly, however, sexual divergence in plasma testosterone is not accompanied by divergence in growth in *S. jarrovii* or in male-larger *C. atrox* in the laboratory. Furthermore, experimental effects of castration and testosterone replacement on growth are not evident in captive *S. jarrovii*, possibly because growth effects of testosterone are superseded by an abundant, high-quality diet. In female-larger *S. undulatus*, growth may be traded-off against testosterone-induced reproductive costs of activity. In male-larger species, costs of reproduction in terms of growth are suggested by supplemental feeding of reproductive female *C. atrox* in their natural environment and by experimental manipulation of reproductive cost in female *S. jarrovii*. Growth costs of reproduction, however, do not contribute substantially to the development of SSD in male-larger *S. jarrovii*. We conclude that the energetic costs of testosterone-induced, male reproductive behavior may contribute substantially to the development of SSD in some female-larger species. However, despite strong evidence that reproductive investment exacts a substantial cost in growth, we do not support the reproductive cost hypothesis as a general explanation of SSD in male-larger species.

Introduction

Body size is one of the most important quantitative traits of an organism because of its pervasive effects on physiological, ecological, and life-history processes. Interspecific differences in body size are thought to reflect, in part, selection for niche diversification. Intraspecific differences in adult body size between sexes (sexual size dimorphism; SSD) may also reflect partitioning of niches between males and females (Butler 2007) but are more often interpreted as evidence for both natural and sexual selection on male and female body size for reproductive advantage (Darwin 1871; Andersson 1994). However, the relative importance of genetic versus environmental control of sexual differences in body size is usually not known (Le Galliard et al. 2006), in part because relatively little is known about proximate physiological mechanisms underlying sex differences in growth (Duvall and Beaupre 1998; Badyaev 2002; Cox and John-Alder 2005; Cox et al. 2005a). Furthermore, most authors have not considered adaptive hypotheses that account for adult size as a correlated response to sex-specific selection for traits other than size itself.

Squamate reptiles (that is, lizards and snakes) historically have served as important models for the study of SSD, due in large part to the considerable variation in both direction and magnitude of SSD observed in this group. The literature is dominated by analyses of adaptive hypotheses that invoke evolutionary processes, but as in other groups of vertebrates, we cannot unambiguously answer the simple question of why adult body size differs between males and females in most species of lizards and snakes. We do not know the relative importance of past evolutionary (that is, genetic) as opposed to current ecological (that is, environmental) processes in the development of SSD, in part because in most species, we do not know how adult SSD develops. Without this information, it is premature to assume that SSD has resulted from selection for body size or size-dependent traits.

In the present report, we briefly consider empirical support for adaptive hypotheses to explain the evolution of SSD in squamates before turning our focus to case studies of environmental and developmental plasticity in body size and potentially in SSD. We synthesize our investigations of both male-larger and female-larger species of Sceloporus (fence and spiny lizards) and the male-larger Crotalus atrox (western diamond-backed rattlesnake) to make the following contributions: (1) Sex-specific growth trajectories in natural populations of animals unequivocally identify sexual differences in growth rate as the developmental cause of SSD; (2) Experiments involving food manipulation and growth of captive animals under "common garden" conditions in the laboratory have demonstrated predominant environmental control of sexual differences in growth and the development of SSD in S. jarrovii and C. atrox (both male-larger); (3) Testosterone may serve as a bipotential endocrine mechanism mediating sexual differences in growth and the development of SSD in both female-larger and male-larger species; (4) Sex-specific trade-offs in allocation of energy between growth and reproduction may cause sexual divergence in growth leading to SSD (that is, "reproductive cost hypothesis"). Integrating these results, we conclude that the energetic costs of testosterone-induced reproductive behavior in males may contribute substantially to the development of SSD in female-larger species (Cox and John-Alder 2005; Cox et al. 2005a). However, despite strong evidence that reproductive investment exacts a substantial cost in growth (Taylor and DeNardo 2005; Cox 2006), we reject the reproductive cost hypothesis as a general explanation of SSD in male-larger species.

Adult body size differs by —10% in the species we investigated, which is enough to provide consistent contrasts. In general, lizards are dominated by male-larger SSD and snakes, even though derived from lizards, by female-larger SSD (Cox et al. 2007).
However, Sceloporus lizards and their parent family Phrynosomatidae are characterized by considerable phylogenetic lability in SSD, where female-larger SSD has evolved apparently independently in Phrynosoma and in three or four clades of Sceloporus. Crotalus atrox and most other species in its parent family Viperidae are unusual among snakes in being characterized by male-larger SSD (Fitch 1981).

Prior to the 2007 SICB symposium titled "Ecological Dimorphisms in Vertebrates: Proximate and Ultimate Causes," one of us (E.N.T. and colleagues) had separately studied energetic, reproductive, and endocrine influences on growth and body size in male-larger C. atrox, while R.M.C. and H.J-A. had begun to develop Sceloporus lizards as a model system for comparative studies on the development of SSD. Our independent research programs had progressed along parallel paths with only occasional consultation, but our convergence on common issues brought us together as authors for that symposium. To some extent, this etiology is reflected in the organization of the present contribution. The general format of the following sections is to present Sceloporus separately from Crotalus, with a synthesis of the two where possible.

Adaptive hypotheses for SSD

Large body size is typically advantageous in agonistic encounters between males, and size can therefore determine access to potential mates and subsequent reproductive success both in lizards and

in snakes (Shine 1994; Cox et al. 2003, 2007). In species with variable clutch sizes, large size in females typically confers a reproductive advantage because the number of eggs or offspring increases with body size. Thus, it is predicted that sexual selection should favor large size in males, while fecundity selection should favor large size in females. It follows that evolutionary increases in aggression and territoriality are predicted to correlate with shifts toward male-larger SSD, while evolutionary shifts in traits associated with fecundity-based selection for large clutch size are predicted to correlate with shifts toward female-larger SSD. As discussed at length by Cox et al. (2007), recent comparative analyses conducted across hundreds of species of lizards and snakes support these predictions as evidenced by correlated evolutionary shifts involving SSD and variables such as aggression by males, territoriality in males, clutch size, and reproductive mode. However, these correlations are generally weak, and comparative support for sexual and fecundity advantage hypotheses for the evolution of SSD is therefore weak.

Why do the predominant adaptive hypotheses not provide satisfactory explanations of interspecific variation in SSD? Weaknesses in these analyses may stem from the use of imprecise categorical proxies for the actual intensity of selection (for example, presence or absence of male combat as an estimate of sexual selection), unaccounted complexity in relationships between body size and reproductive success (Baird et al. 1997; Lappin and Husak 2005) and the possibility that selection for body size may not always be directional (for example, Calsbeek and Sinervo 2004). A larger issue, however, may be the common failure to consider the evolution of size in a broader selective context. The magnitude of SSD will depend not only on the strength of selection on male and female body size themselves but also on factors that influence the complex growth trajectory leading to adult size. For example, selection for traits other than size that confer reproductive advantage (for example, reproductive investment) could force sex-specific trade-offs in allocation of energy that differentially impact growth both in males and in females. If such energetic growth constraints are prevalent in squamate reptiles, this may help to explain why adaptive hypotheses that invoke sexual selection and fecundity selection have so little explanatory power in broad-scale comparative analyses.

Development of SSD

Sexual size dimorphism in natural populations can reflect multiple contributing factors in place of, or in addition to, sexual differences in age-specific body size (Stamps 1993; Watkins 1996). For example, sexual differences in the body size of sampled individuals can reflect sexual differences in size at birth, prematurational or post-maturational growth trajectories, survival, emigration and recruitment, behavioral exclusion of small individuals, or some combination of these factors (Stamps 1993; Watkins, 1996; Haenel and John-Alder 2002). To understand both how and why SSD arises, it is essential to determine which of these factors contribute to SSD within a population. Badyaev (2002) recently emphasized the importance of an ontogenetic perspective in which SSD is viewed as a developmental process of "growing apart." We have determined the origin of SSD in our studies by characterizing sex-specific growth trajectories through mark-recapture studies of individuals of known age in their natural environments. This demographic approach has ruled out sexual differences in mortality and migration as a significant
contributing cause of SSD (Haenel and John-Alder 2002; Cox and John-Alder 2007a). Moreover, our descriptions of the development of SSD have identified critical periods in which male and female sizes diverge, thereby suggesting testable hypotheses for proximate determinants of SSD (see below).

In *Sceloporus* lizards, we have characterized sexual differences in growth by fitting asymptotic growth curves to recapture data and by measuring linear growth over discrete time intervals for individuals of known age (Fig. 1). The first approach accounts for non-linear patterns of growth as a

function of age and allows us to explicitly test for sexual differences in model parameters such as asymptotic size. The second approach reveals the discrete ontogenetic periods when the sexes differ in growth rate, thus identifying the appropriate timing for subsequent experiments addressing the causal mechanisms for divergence of growth between the sexes.

Despite notable differences in growth patterns among species of *Sceloporus* and between *Sceloporus* and *C. atrox,* SSD arises because of sexual differences in age-specific growth rates in all cases (Fig. 1). In *Sceloporus*, body size is indistinguishable between neonatal males and females, but SSD [as described by the index of Lovich and Gibbons (1992)] develops to the magnitude characteristic of populations of adults by ~1 year of age, well before the attainment of asymptotic body size. In *S. undulatus* and *S. virgatus* (both female-larger), SSD develops because females grow faster than males as sexual differences in reproductive roles begin to emerge. In *S. jarrovii* (male-larger), males grow faster than females from the onset of neonatal life, and SSD develops primarily during the first year of post-natal life (Cox and John-Alder 2007a). In *C. atrox*, males and females are equal in size as neonates and grow at similar rates until first reproduction. Significant SSD does not arise until reproductive maturity (Beaupre et al. 1998; Taylor and DeNardo 2005). Beyond this point, males grow more than twice as fast and become appreciably larger than females (Taylor and DeNardo 2007).

Environmental sensitivity of growth and SSD

Field studies on sex-specific growth trajectories coupled with demographic analyses of survivorship and age distributions have identified sexual divergence in growth as the proximate cause of SSD (Fig. 1). Sexual differences in growth indicate a difference in energy available for or allocated to growth in males and females. To address the issue of whether these energetic differences are controlled predominantly by genetic or environmental factors, we compared growth in the field versus laboratory common garden conditions to provide initial characterizations of the reaction norms of growth rate. Experiments discussed below indicate that growth rate is strongly responsive to the availability of food and to other proximate environmental conditions, and sex differences in growth rate can be muted or even absent in animals raised under favorable growth conditions in the laboratory (Fig. 2). These findings indicate that growth rate is not genetically fixed in females and males and that SSD itself cannot be entirely due to genetic effects on the allocation of energy to growth.

Haenel and John-Alder (2002) compared growth of laboratory-hatched, female-*larger S. undulatus* during the first 4 weeks of post-natal life to that of field-active juveniles within the size range defined by the laboratory cohort. This comparison involved a laboratory cohort growing in August and September immediately after hatching versus a field cohort growing in March through June after hatching in August or September of the previous year. In this experiment, growth rate did not differ between the sexes in the laboratory. Males grew as fast as females in the laboratory and considerably faster than similarly sized males in the field, while females grew almost equally fast in the field and the laboratory. Thus, consistent with an earlier report (Ferguson and Talent 1993), growth *in S. undulatus* is highly responsive to environmental conditions. A subset of hatchlings was retained in the laboratory for longer-term analysis of growth to the size of maturity. Females in this group began to grow faster and became larger on average than males (Haenel and John-Alder 2002; Fig. 2A). Even though the emerging sexual difference in growth rate failed to achieve statistical significance, this result may indicate that the development of SSD was delayed,before allowing a firm conclusion regarding the eventual development of significant SSD.

Experiments on *S. jarrovii* (male-larger) provide stronger evidence for predominant environmental control of sex differences in growth and body size (Cox et al. 2006). Growth rates of female and male *S. jarrovii* yearlings are statistically indistinguishable and SSD does not develop under favorable, common-

garden laboratory conditions even up to the size at which SSD is fully developed in the wild (Fig. 2B). This experiment was conducted for a sufficiently long time during appropriate developmental stages to support relevant comparisons of growth and the development of SSD between laboratory and field. The results therefore indicate a predominant environmental influence not only on growth but also on the development of SSD. Our findings corroborate an earlier report of substantial environmental plasticity in growth of yearling *S. jarrovii* (Smith et al. 1994). The similarity in growth rate between captive male and female *S. jarrovii* yearlings in our laboratory appears to have been caused by a reduction in growth rate of captive males compared to growth in the field, indicating that the high growth rate of field-active yearling males depends on factors in the natural environmental milieu. Subsequent experiments demonstrated that sexual differences in growth are nearly absent either on an *ad libitum* diet or at 1/3 ration, despite a 2-fold difference in growth between lizards of the same sex on different diets (Cox et al., unpublished data). Thus, in the absence of the ecological context for sexual differences in energy acquisition and/or allocation, sexual differences in growth rate are not expressed in *S. jarrovii*. In a similar experiment on the European Common Lizard (*Lacerta vivipara*), Le Galliard et al. (2005) reported that growth rate is equally susceptible to food restriction in juvenile males and females.

Crotalus atrox (male-larger) appears similar *to S. jarrovii* in that growth is highly responsive to the availability of food (Taylor et al. 2005), and natural sexual differences in growth and body size can be completely eliminated in captivity (Fig. 2C; Taylor and DeNardo 2005). In the field, both growth rate and reproductive frequency are substantially increased by supplemental feeding of mature females (Taylor et al. 2005), although it remains to be seen whether natural sexual differences in growth and the development of SSD could have been experimentally eliminated by supplemental feeding. In the laboratory, growth rates of female and male *C. atrox* neonates are substantially higher than in the field on either a high (one rodent meal per week) or a low (one rodent meal every 3 weeks) ration (Taylor and DeNardo 2005). Sexual differences in growth and body size are completely absent even at reproductive maturity under these conditions. Although snakes with high intake of food had begun to reproduce, SSD was not apparent even after 2 years of growth in the laboratory, well beyond the body size at which SSD develops in the field. These experiments suggest the absence of an inherent sex difference in growth in *C. atrox* and indicate that the growth cost of reproduction in females can be compensated by supplemental feeding. The absence of sex differences in growth in captive *C. atrox* even when food intake is restricted suggests that other demands on energy output are sufficiently high to force a reduction in growth only in field-active females.

Testosterone and sex-specific regulation of growth

To minimize intersexual genetic conflict, males and females are predicted to share most of the basic genetic components underlying the regulation of growth. Sexual differences in growth are predicted to result from epigenetic interactions with sex-specific regulators (Badyaev 2002). Sex steroids are obvious candidates for sex-specific growth regulation because they are differentially produced and secreted in males and females. Testosterone is commonly regarded as an anabolic steroid that promotes growth, but most of the evidence supporting this generalization comes from species with male-larger SSD (reviewed by Cox and John-Alder 2005). Interestingly, several isolated reports hint that testosterone may inhibit growth in species with female-larger SSD (Swanson 1967; Sockman and Schwabl 2000; Sockman et al. 2005). This raises the intriguing possibility that testosterone may act as a biopotential growth regulator, thereby providing a common endocrine mechanism for the development of opposite patterns of SSD. Squamates provide an ideal system in which to test this hypothesis by characterizing the effects of testosterone on growth in closely related species with opposite patterns of SSD.

In *S. undulatus*, SSD develops because males grow more slowly than females in conjunction with maturational increases in home range area and territorial behavior of males, and in male-specific coloration (Skelly and John-Alder 2002), traits known to depend at least partially on testosterone (Marler and Moore 1989, 1991; Smith and John-Alder 1999; Quinn and Hews 2003; Klukowski et al. 2004; Cox et al. 2005b). In *S. virgatus*, SSD develops because males grow more slowly than females in conjunction with first reproduction. These natural historical contexts suggest that sexual divergence in growth in both species occurs during periods when males and females diverge in plasma testosterone levels. Our characterization of the ontogeny of sexual divergence in plasma testosterone confirms this conjecture. In both species, plasma testosterone becomes markedly higher in males than in females in association with sexual divergence in growth rate (Fig. 3; Cox and John-Alder 2005; Cox et al. 2005a), implicating plasma testosterone as a key mediator of sexual divergence in growth and the development of SSD. *S. jarrovii* exhibits a nearly identical sexual divergence in plasma testosterone during the first breeding season, but throughout the first year of life males of this species consistently grow more quickly than do females. Together, these observations raise the possibility that testosterone may act as a bipotential epigenetic regulator of SSD by stimulating growth of males in male-larger *S. jarrovii* while inhibiting males' growth in female-larger *S. undulatus* and *S. virgatus.*

We conducted experiments on field-active lizards to test the hypotheses that testosterone inhibits growth in yearling males of *S. undulatus* and *S. virgatus* while stimulating growth in yearling males of *S. jarrovii* (Cox and John-Alder 2005; Cox et al. 2005a). These experiments were conducted during critical periods in the development of SSD marked by natural peaks in male testosterone and maximal sexual divergence in growth rate, as revealed by our demographic analyses of growth in known individuals over discrete time periods (Fig. 3). The basic design was similar in all experiments: treatments involved (1) sham surgery, (2) surgical castration to remove the primary endogenous source of testosterone, and (3) castration accompanied by replacement of testosterone via an intraperitoneal Silastic® tubule containing 300 μg of testosterone (Cox and John-Alder 2005). These tubules maintained plasma testosterone in the midrange of a reference group of free-living, same-aged males. *S. virgatus* and *S. jarrovii* males were released after surgery at their sites of capture, while experiments on *S. undulatus* were replicated on separate cohorts of lizards during consecutive summers inside an enclosed tract of natural habitat at the Rutgers Pinelands Research Station.

In both of the female-larger species *(S. undulatus* and *S. virgatus*), testosterone replacement reduced growth rate in castrated males (Fig. 4). In the male-larger species (*S. jarrovii*), the effects were opposite: castration caused a reduction in growth rate, and testosterone replacement restored growth rate to that of controls. Mean growth rate did not differ between castrated males and intact control males in either of the two female-larger species. However, two lines of evidence indicate that castration actually promotes growth of males in both of these species. In *S. undulatus*, the stimulatory effect of castration on growth did not attain statistical significance during the immediate 2- month period following manipulation, but it was readily apparent in animals recaptured the following summer (Cox et al. 2005a). The short-term ineffectiveness of surgical castration in that experiment may be related to the fact that plasma testosterone was unusually low in intact experimental males and that surgical castration did not immediately result in a further reduction in plasma testosterone (Cox et al. 2005a).

In *S. virgatus*, the stimulatory effect of castration on growth was evident in large but not small yearling males (Cox and John-Alder 2005). The explanation for this treatment-by-size interaction stems from the observation that plasma testosterone is higher in large than in small yearling *S. virgatus* males and is positively correlated with body size. Thus, surgical castration likely had little effect on plasma testosterone in small lizards with naturally low plasma testosterone, while the effect in larger lizards was substantial. It follows that if testosterone were to influence growth in experimental groups relative to controls, then the stimulatory effect of surgical castration on growth would be apparent in large but not in small yearling males, while the growth-inhibitory effect of testosterone replacement would be

greater in small than in large yearlings. Accordingly, the treatment-by-size interaction corroborates the experimental result that castration increased growth in large yearlings but had no effect in small yearlings relative to controls. This predicted size disparity in responsiveness to testosterone was not evident in castrates because plasma testosterone was uniformly low in castrates. Testosterone replacement was therefore effective in inhibiting growth regardless of body size.

In striking contrast to the experimental effects in the two female-larger species, growth rate was reduced by surgical castration and restored by testosterone replacement in male-larger *S. jarrovii* (Cox and John-Alder 2005). This is the first unequivocal evidence that growth can be promoted by testosterone in any squamate (Crews et al. 1985; Hews et al. 1994; Abell 1998a; Klukowski et al. 1998; Lerner and Mason 2001; Uller and Olsson 2003; Cox
and John-Alder 2005; Cox et al. 2005a). This important difference in the growth-regulatory effects of testosterone in *S. undulatus* and *S. virgatus* versus *S. jarrovii* depended on several critical elements of experimental design, including: (1) precision-loading of Silastic® implants with small quantities of testosterone (Cox and John-Alder 2005), (2) the use of relevant natural history in the scheduling of experiments, (3) the return of experimentally manipulated lizards to their natural habitat, and (4) the inclusion of surgical castration and testosterone replacement in separate treatment groups.

Experiments summarized here report the first direct evidence that testosterone can act as a bipotential regulator of growth of males in closely related species with opposite patterns of SSD. This finding raises the possibility that testosterone may generally be either stimulatory or inhibitory to growth in *Sceloporus* and other organisms, depending on the pattern by which differential growth leads to the development of SSD. In other classes of vertebrates, testosterone is generally considered to be a growth-promoting anabolic steroid (Ford and Klindt 1989; Borski et al. 1996; Gatford et al. 1998; Holloway and Leatherland 1998). Most previous work on mammals, birds, and fishes, however, has involved male-larger species, although two isolated reports suggest that testosterone may be bipotential even in those classes of vertebrates. Swanson (1967) reported that castration promotes growth in female-larger golden hamsters, implying that testosterone itself may inhibit growth in this species, and Sockman and colleagues (Sockman and Schwabl 2000; Sockman et al. 2005) reported that injection of testosterone into yolk of incubating eggs reduces post-natal growth of males in female-larger American kestrels.

Effects of testosterone on growth in snakes are less clear than in lizards. The pioneering study of Crews et al. (1985) is widely cited as providing evidence that testosterone inhibits growth in male garter snakes (*Thamnophis sirtalis parieatlis*) (Abell 1998a, p 534; Shine and Crews 1988, p 1105). However, neither surgical castration nor testosterone replacement influenced male growth as measured by changes in body length, the preferred measure of progressive growth in reptiles (Andrews 1982), and the authors of the study concluded that “further work is required to determine if body growth in the red-sided garter snake can be influenced by sex steroids during development” (Crews et al. 1985). A subsequent investigation of the effects of sex steroids on growth in *T. sirtalis* was marked by concern over excessively high experimental levels of testosterone (Lerner and Mason 2001, p 223).

Attempts to investigate potential effects of testosterone on growth in *C. atrox* have been only partially successful (Taylor and DeNardo 2007). Adult males were surgically castrated, released in the field, and recaptured periodically over a period of —15 months for measuring their weight and length and for collecting blood samples. Castrated snakes tended to grow faster in both mass and length than did controls, although these tendencies did not attain statistical significance, and at the end of the experiment, castrated snakes had significantly heavier abdominal fat bodies and significantly higher percent body fat. Unexpectedly, however, castrated snakes continued to have substantial amounts of plasma testosterone, possibly from an unspecified extratesticular source, and the interpretation of results is therefore problematic.

Relationships between sexual divergence in plasma testosterone and growth rate are not apparent under conditions favorable for growth in the laboratory. In *C. atrox* and *S. jarrovii* (both male-larger), maturtional sexual divergence in plasma testosterone is unaccompanied by sexual differences in growth rate in captive animals (Taylor and DeNardo 2005; Cox et al. unpublished). Thus, testosterone may not be functionally related to sexual differences in growth, or the growth effects of testosterone may be sensitive to environmental differences between field and laboratory, just as are natural sexual differences in growth. For example, growth effects of testosterone may be overshadowed by high food availability in the absence of an ecological context for energetic trade-offs.

Whatever the explanation, the discrepancy between captive and field-active animals exemplifies limitations of correlational observations and emphasizes the necessity of experimental approaches. However, experimental responses to castration and testosterone are strongly dependent on environmental conditions. In two experiments on captive yearling *S. jarrovii* males of the same age as those we studied in the field, we were unable to detect inhibition of growth by castration or stimulation by testosterone replacement under conditions favorable for growth in the laboratory (Cox et al. 2006). In these experiments, treatment effects on plasma testosterone were comparable between the laboratory and the field. Furthermore, hormonal responses to experimental manipulations in the laboratory were not without physiological effect: for example, castration reduced the intensity of gular pigmentation and testosterone replacement increased it. Thus, natural sexual differences in growth as well as growth responses to endocrine manipulation can be expressed differently in the field versus the laboratory. Experiments must be carried out on animals active in their natural environment.

Costs of reproduction to growth

How can testosterone have opposite effects on growth in female-larger versus male-larger species? An obvious possibility is that testosterone may act as a bipotential "switch" to facilitate the sex-specific regulation of a shared endocrine growth axis, thereby minimizing intersexual genetic conflict while producing different adaptive patterns of growth and SSD in different species (Badyaev 2002). Under this scenario, testosterone would be predicted to stimulate growth-promoting functions of the somatotrophic axis in male-larger species while inhibiting these functions in female-larger species (John-Alder and Cox 2007). The somatotrophic axis is the central endocrine axis involving pituitary growth hormone and hepatic insulin-like growth factor I that regulates somatic growth in vertebrates (Duan 1997). It is well established in mammals and fishes that growth and the growth-promoting functions of the somatotrophic axis are enhanced by testosterone and other androgenic steroids, while estrogenic hormones often have the opposite effect (Jansson et al. 1985; Eden et al. 1987; Millard et al. 1987; Devesa et al. 1991; Painson et al. 1992; Borski et al. 1996; Pincus et al. 1996; Riley et al. 2002a, 2002b; Sparks et al. 2003; Larsen et al. 2004; Arsenault et al. 2004). Thus, as summarized by Gatford et al. (1998) *"the somatotrophic axis may be a major pathway through which steroids act to produce sex differences in growth.*" One can easily imagine that testosterone could inhibit growth-promoting functions of the somatotrophic axis (directly or via aromatization to estrogens) in female-larger species, an issue we are currently investigating in *Sceloporus*.

An alternative possibility is that testosterone may actually stimulate growth-promoting functions of the somatotrophic axis in both male-larger and female-larger species, as might be expected from the generalization that testosterone is an anabolic steroid. Despite the fact that yearling males grow more slowly than do females in *S. undulatus* and *S. virgatus*, large size confers an advantage when males of these species compete for access to mates (Vinegar 1975; Smith 1985; Haenel et al. 2003a), and male mating success is itself correlated with body size (Abell 1998b; Haenel et al. 2003b; Haenel and John-Alder, unpublished). It therefore seems unlikely that selection for small male size per se would have led

to the evolution of a mechanism by which testosterone inhibits growth while promoting other correlates of reproductive success. Instead, androgenic stimulation of the somatotrophic axis may be conserved regardless of sexual differences in organismal growth, and the effects of testosterone on organismal growth may be secondary to other effects of testosterone.

In addition to its direct effects on growth, testosterone also stimulates energetically costly reproductive activities, and in female-larger species such as *S. virgatus* and *S. undulatus*, energy may be allocated to these activities at the expense of growth (Riley et al. 2003). Indeed, we have previously shown that exogenous testosterone increases daily activity period, movement, and home range area in males of *S. undulatus* (Fig. 5) (Cox et al. 2005a), and our estimates indicate that the increased energy requirements of these behaviors can account for at least 80% of the reduction in growth rate induced by testosterone. Thus, even while testosterone promotes growth through the somatotrophic axis, it may indirectly inhibit organismal growth in some species due to energetic trade-offs with reproductive investment. This trade-off in allocation of energy may be further exacerbated by additional costs of testosterone-induced ectoparasitism (Cox et al. 2005a; Cox and John-Alder 2007). Interspecific differences in the growth effect of testosterone may be related to life-history variation in the relative energetic demands of competing functions stimulated by testosterone and the balance between total organismal energetic demands and the environmental availability of energy due to differences in habitat and/or breeding phenology. This conjecture is discussed more fully by Cox and John-Alder (2005, p 4685) and will not be elaborated here. In any case, if testosterone is generally stimulatory with regard to the somatotrophic axis while having an opposite effect on organismal growth, then clearly the life histories of male-larger species such as *S. jarrovii* must somehow differ from those of *S. undulatus and S. virgatus* for testosterone-induced energetic costs not to detract from growth.

While the inhibition of growth by testosterone may be largely attributed to growth costs of energetic investment in reproductive activity, at least in female-larger species, any complete explanation of SSD must consider growth patterns and body size not just in males but also in females. In male-larger *C. atrox*, supplemental feeding greatly increases growth in reproductive females (Taylor et al. 2005), and sexual differences in growth are absent when captive snakes are raised on controlled diets (Taylor and DeNardo 2005). These findings suggest that growth costs of female reproduction may predominate in the development of SSD when energy is limited. Indeed, studies on energy expenditure of rattlesnakes concur that the energetic cost of reproduction in adult females contributes to their decreased growth relative to that of males (Beaupre and Duvall 1998; Beaupre 2002). In male-larger *Cophosaurus texanus*, estimates of the growth cost of egg production suggest that females would grow as fast as males and that SSD would be eliminated if females were to allocate the energy content of a clutch of eggs into growth (Sugg et al. 1995). However, while these studies provide strong evidence for substantial growth costs of reproduction in females of male-larger species, implications regarding the development of SSD are inferential.

Cox (2006) investigated the contribution of growth costs of reproduction through comparative and experimental studies on *S. jarrovii*. Within populations of this species from low elevations, many but not all females reproduce as 1-year olds. SSD develops in part during this first reproductive season, suggesting that growth costs of female reproductive investment may explain the development of male-larger SSD. Comparisons of both natural and experimentally induced variation in reproductive status indicate a growth cost of female reproduction (Fig. 6). Growth is slower in females that reproduce as yearlings than it is in those that fail to reproduce, both during pregnancy and for several months after parturition. However, this difference in growth can be attributed to differences in initial body size without invoking reproductive status. A direct experimental comparison of females that were ovariectomized to remove reproductive costs versus size-matched sham controls revealed that the experimental group had a similar cost of growth during pregnancy and following parturition. However, the increase in growth observed among non-reproductive females in either case can only account for a

small fraction of SSD. Comparisons between populations also indicate that SSD develops even in the absence of female reproductive costs. While females in populations from low elevations commonly reproduce as 1-year olds, females from populations at high elevations delay reproduction until their second year. Despite this clear contrast in reproductive phenology, SSD develops in both populations by 1 year of age. Although growth costs of reproduction can clearly be demonstrated in *S. jarrovii* females, these costs have little bearing on the development of SSD in this male-larger species.

Concluding remarks

Sexual selection and natural selection for body sizes of males and females have clearly contributed to patterns of SSD in extant squamates, but sexual differences in growth leading to SSD can be influenced predominantly by environmental factors rather than genetic ones. We have identified testosterone as a likely epigenetic factor for sex-specific regulation of growth, whereby testosterone inhibits growth of males in female-larger species and stimulates growth of males in male-larger species. At this point, we can only speculate about underlying mechanisms of these divergent growth responses, but our results raise interesting questions about the role of testosterone in promoting sexual differences in growth and body size. Characterization of the molecular endocrinology of the regulation of growth will undoubtedly be useful in this regard. One possibility is that testosterone has fundamentally different effects on the somatotrophic axis in male-larger versus female-larger species. Alternatively, testosterone may enhance growth-stimulatory functions of the somatotrophic axis (the endocrine growth axis) in both groups. If this is true, energetic costs of testosterone-induced reproductive activity may be traded-off against growth and may limit body size in males of some species. The diversity of SSD in Sceloporus makes this group well suited for further comparative studies of this issue.

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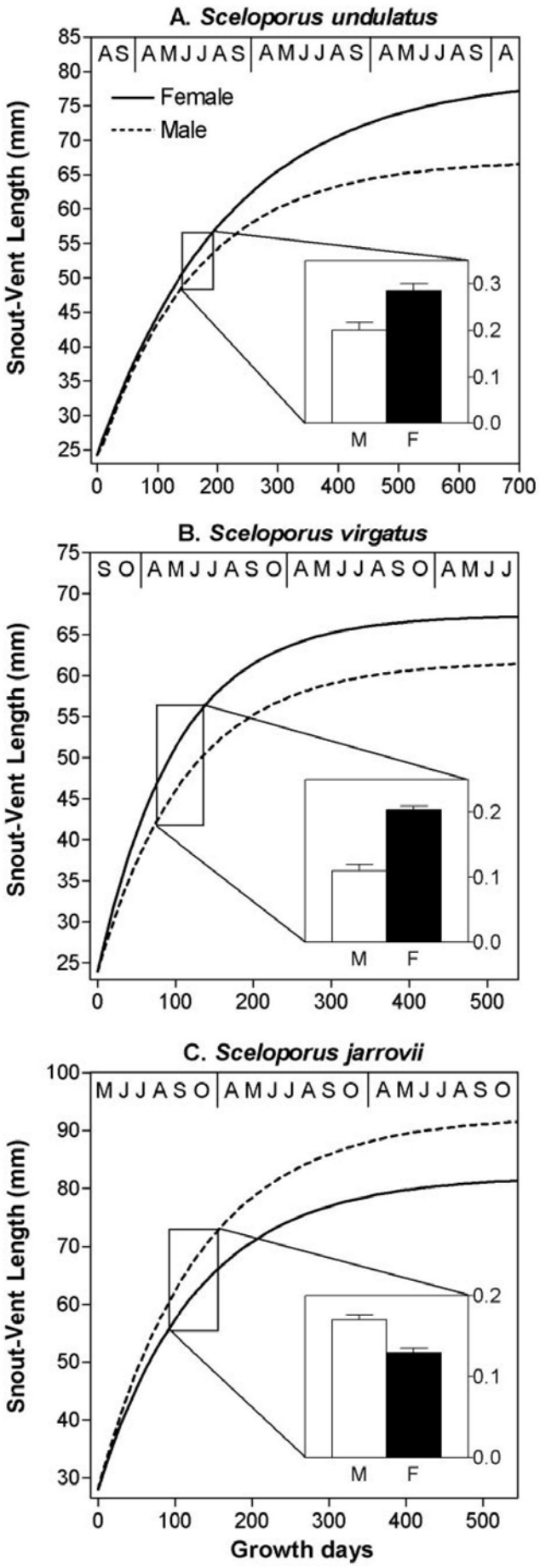

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Fig. 1 Growth trajectories of males and females derived from the interval form of the von Bertalanffy growth equation using recapture data from three *Sceloporus* species. Inserts show linear growth rates (mm/day) for each sex during critical periods in the development of SSD. In all species, non-linear models revealed a significant sexual difference in asymptotic size, and linear growth rates verified sexual differences in absolute growth rate as the source of this SSD. Letters along the top of each panel indicate months so as to provide ontogenetic scaling of the growth trajectories. Periods of winter dormancy are omitted for clarity. See Cox (2006) for analyses of asymptotic growth. Redrawn from data in papers by Haenel and John-Alder (2002), Cox (2006), and Cox and John-Alder (2007a).

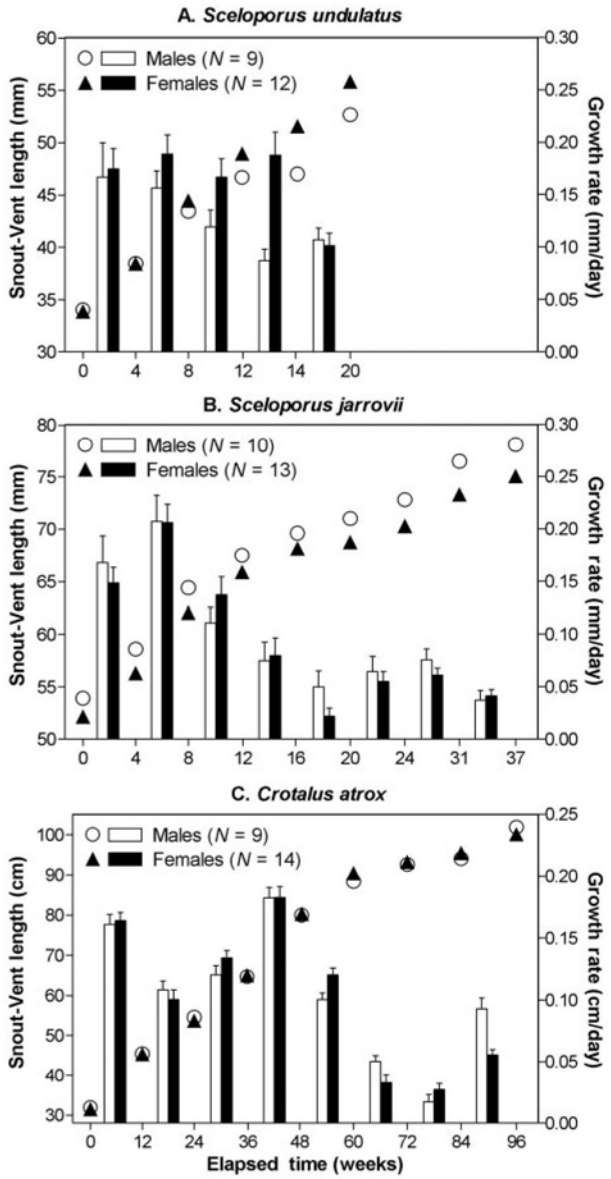

Fig. 2 Mean (±1 SE) growth rate (bars) and snout-vent length (SVL) (circles and triangles) over time for males and females of three squamate species raised under common-garden laboratory conditions. *Sceloporus undulatus* (A) *and C. atrox* (C) neonates were raised from birth, at which point males and females did not differ in size. *Sceloporus jarrovii* (B) juveniles were transplanted to the laboratory at ~2–3 months of age, at which time the sexes had already begun to diverge in size. In both *S. jarrovii* and *C. atrox*, sexual differences in growth and the development of SSD were suppressed in the laboratory. Data are shown for animals raised on high-intake diets, although the development of SSD was also suppressed in *S. jarrovii* and *C. atrox* raised on low-intake diets. Redrawn from data in papers by Haenel and John-Alder (2002), Taylor and DeNardo (2005), and Cox et al. (2006).

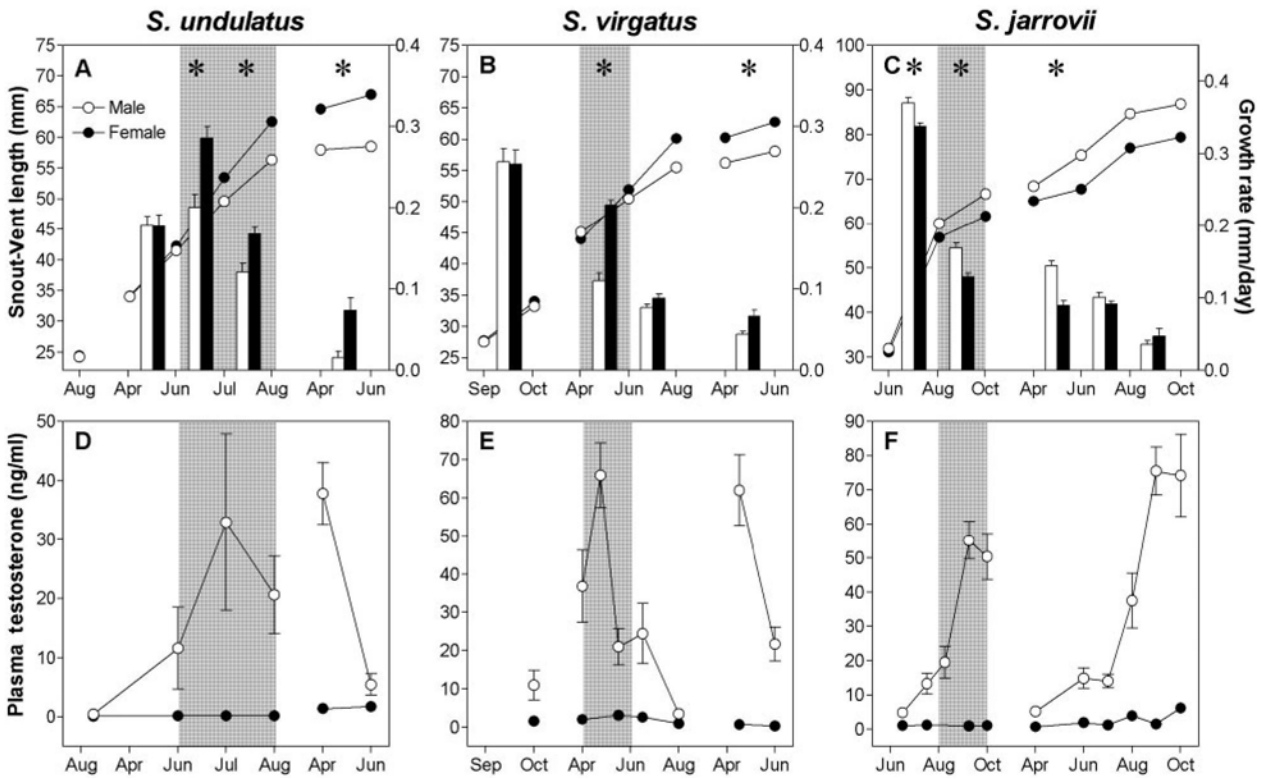

Fig. 3 Development of sexual differences in growth rate and SVL (A–C) and plasma testosterone levels (D–F) for three *Sceloporus* species. Data are means (± 1 SE) for free-living males (open symbols) and females (filled symbols) of known age. Asterisks indicate significant sexual differences in growth rate (bars), which give rise to sexual dimorphism in SVL (circles). Breaks in connecting lines indicate periods of winter dormancy. In the two female-larger species *(S. undulatus* and *S. virgatus*), sexual differences in growth rate correspond to seasonal peaks in male plasma testosterone levels. By contrast, yearling males of *S. jarrovii* grow more quickly than do females regardless of seasonal changes in plasma testosterone. Shaded areas indicate the timing of subsequent manipulations of testosterone planned to coincide with sexual divergence in growth rate and plasma testosterone (Fig. 4). Redrawn from data in papers by Haenel and John-Alder (2002), Cox et al. (2005), Cox and John-Alder (2005), and Cox and John-Alder (2007a).

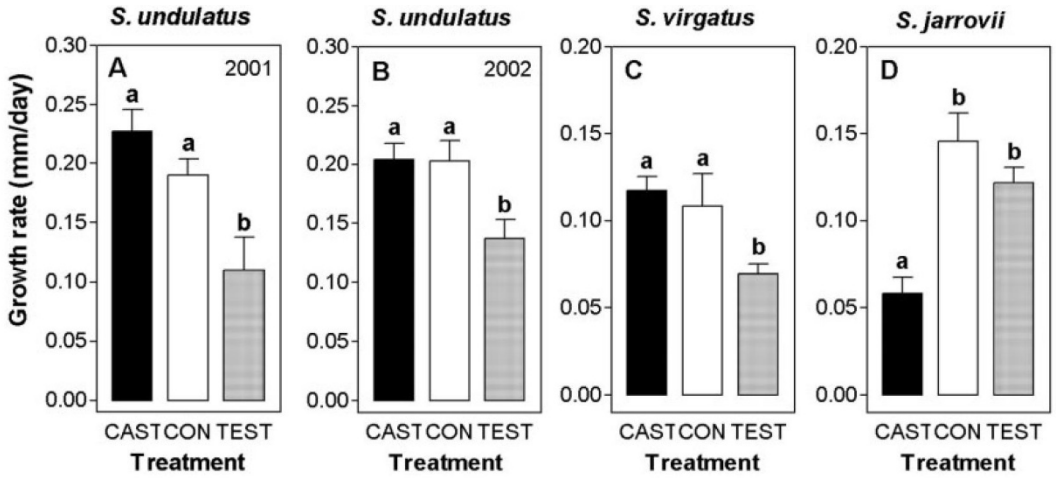

Fig. 4 Mean (± 1 SE) growth rate over the 6–8-week period following surgical castration and treatment with exogenous testosterone for yearling males of three *Sceloporus* species in their natural field environments. CAST = surgical castration+ empty implant; CON =control sham surgery + empty implant; TEST = surgical castration+ testosterone implant. This experiment was replicated in two separate years for S. undulatus. Experiments were carefully designed to coincide with natural sexual divergence in growth rate and seasonal peaks in yearling male plasma testosterone levels (Fig. 3). Lowercase letters denote statistical separation among treatment groups. In the two species with female-biased SSD *(S. undulatus* and *S. virgatus*), testosterone strongly inhibits growth in males. By contrast, castration inhibits growth in male-larger *S. jarrovii*, while exogenous testosterone restores growth of castrated males to the rate found in intact controls. Redrawn from Cox et al. (2005) and Cox and John-Alder (2005).

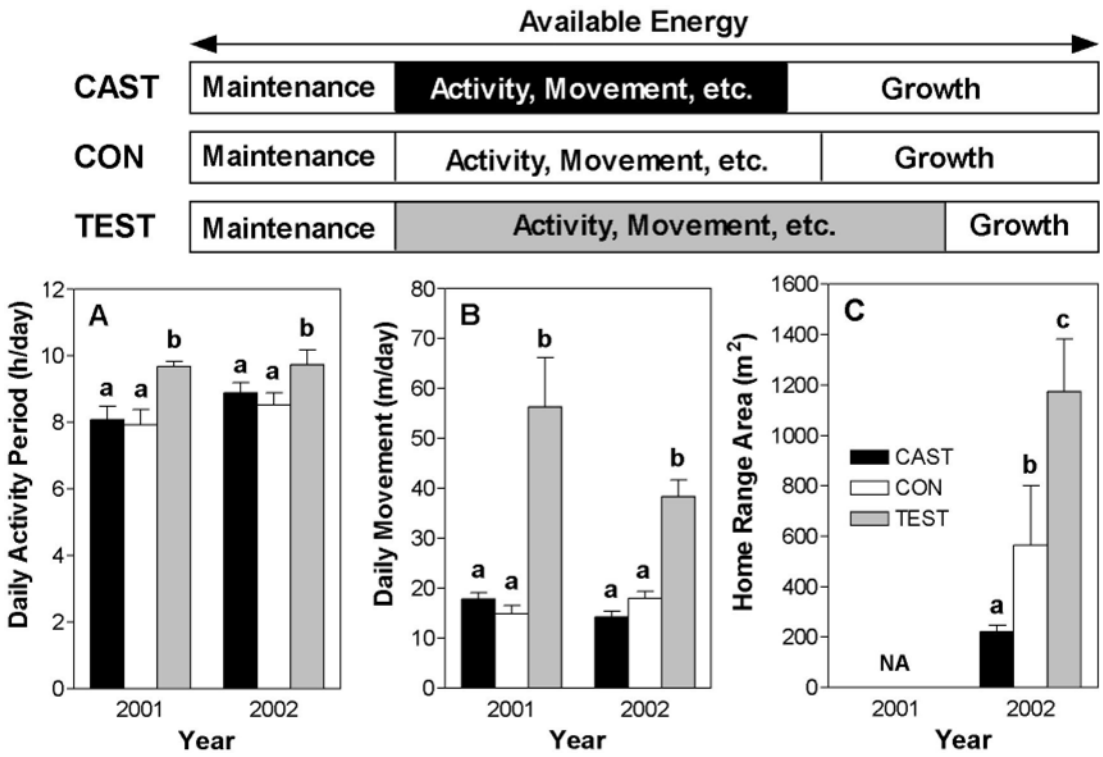

Fig. 5 Testosterone increases (A) daily activity period, (B) daily movement, and (C) home range area of *S. undulatus* males. Data are treatment means ± 1 SE. Lowercase letters denote statistical separation of treatment groups. See Fig. 4 for explanation of treatment group abbreviations. This experiment was replicated in two separate years, although home range areas were calculated only for the second year. Energetic costs of increased activity, movement, and home range area may explain why testosterone inhibits growth in this species. Redrawn from data provided by Cox et al. (2005a).

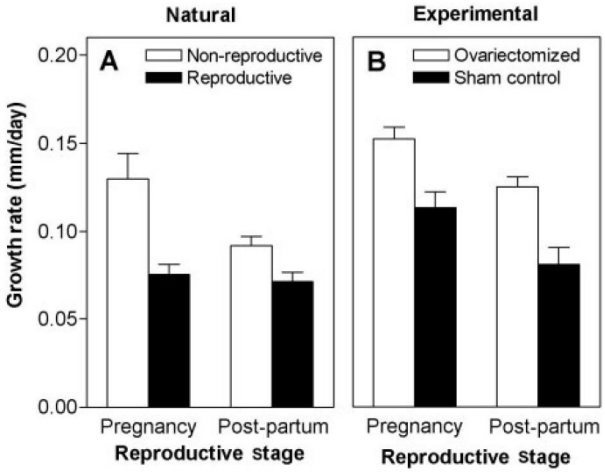

Fig. 6 Mean (±1 SE) growth rates for *S. jarrovii* females that differed in reproductive status, either naturally (A) or experimentally due to surgical ovariectomy (B). For all comparisons, non-reproductive females grew more quickly than did reproductive females during pregnancy (May–June) and for several months following parturition (June–August). These correlative and experimental data indicate a growth cost of female reproduction, but several independent lines of evidence suggest that this cost is insufficient as an explanation for male-larger SSD. See text and Cox (2006) for details. Redrawn from Cox (2006).